

Exhibit 3



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 1: Oral Dis. 2002 Nov;8(6):275-81.


Links

Salivary gland delivery of pDNA-cationic lipoplexes elicits systemic immune responses.

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OBJECTIVE: To test the ability of two cationic lipoplexes, Vaxfectin and GAP-DLRIE/DOPE, to facilitate transfection and elicit immune responses from plasmid DNAs (pDNAs) after retrograde instillation into salivary glands. **METHODS:** Two pDNA expression vectors encoding either the influenza NP protein or human growth hormone (hGH) were complexed with the cationic lipid transfection reagents, GAP-DLRIE/DOPE or Vaxfectin, and delivered to the submandibular glands of rats. Samples from rats receiving the influenza NP protein pDNA and cationic lipoplexes were analyzed for anti-influenza NP-specific IgG1, IgG2a, and IgG2b in serum, and IgA in saliva, by an enzyme-linked immunosorbent assay (ELISA). Cytotoxic T-cell lymphocyte (CTL) assays were also performed. Transgene protein expression (hGH) was determined from extracts of submandibular glands of rats receiving hGH lipoplexes. **RESULTS:** Serum antibodies (IgG) against the NP protein developed and were highest in all rats vaccinated with GAP-DLRIE/DOPE or Vaxfectin. The major serum IgG subclass stimulated by this route of immunization was IgG2b, followed by IgG2a. CTL assay results showed statistically significantly higher percentage killing in the Vaxfectin group than controls ($P < 0.05$). No rats developed IgA antibodies to NP protein in saliva. Animals receiving plasmid encoding hGH and either lipoplex expressed significantly higher amounts of hGH compared with those receiving the hGH plasmid and water. Although hGH expression was higher in the animals receiving pDNA/Vaxfectin (approximately 30-fold > pDNA/water), the difference with those receiving pDNA/GAP-DLRIE/DOPE (approximately 10-fold > pDNA/water) was not significant. **CONCLUSIONS:** Retrograde instillation of pDNA complexed with Vaxfectin into the salivary glands can stimulate cytotoxic and humoral responses to the influenza NP protein antigen.

Related Links

- Vaxfectin enhances the humoral immune response to plasmid DNA-encoded antigens. [Vaccine. 2001]
- Cationic liposome-mediated gene transfer to rat salivary epithelial cells in vitro and in vivo. [J Gene Med. 2001]
- Improved tuberculosis DNA vaccines by formulation in cationic lipids. [Infect Immun. 2002]
- Vaxfectin enhances antigen specific antibody titers and maintains Th1 type immune responses to plasmid DNA immunization. [Vaccine. 2001]
- Targeted salivary gland immunization with plasmid DNA elicits specific salivary immunoglobulin A and G antibodies and serum immunoglobulin G antibodies in mice [Infect Immun. 1999]

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Optimization of the conditions required to stimulate humoral and secretory antibody formation may facilitate use of this tissue for specific clinical applications of pDNA immunization.

PMID: 12477057 [PubMed - indexed for MEDLINE]

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